

Elucidating Bioreductive Transformations within Physically Complex Media: Impact on the Fate and Transport of Uranium and Chromium ERKP493

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ORNL project: Elucidating Biogeochemical Reduction of Chromate via Carbon Amendments and Soil Sterilization

Rationale: Contaminated subsurface environments at many DOE facilities contain legacy hexavalent Cr(VI) bearing waste. The oxidized hexavalent Cr(VI) species is toxic, carcinogenic, and teratogenic whereas the reduced trivalent Cr(III) species is an essential trace element. In the oxidized state, the most prevalent form of chromium (CrO_4^{2-}) is anionic and therefore more bioavailable than the cationic Cr(III). Cr(VI) is highly mobile due to its solubility as an oxyanion and Cr(III) is only sparingly soluble at pH >4. Transformation of toxic and mobile Cr(VI) to innocuous and immobile Cr(III) is a desirable form of contaminant remediation and it may be achieved *in situ* at some contaminated sites.

Objectives: The greatest challenge to elucidating geochemical and biological chromium reduction in natural sediments is to create a sterile environment without destroying the chemical and physical properties of the system. In this study we determined the potential for geochemical and biological chromium reduction in a naturally reducing soil using carbon amendments and sterilization. To minimize alterations to the sediment samples, soils were sterilized via exposure to γ -irradiation which causes fewer changes in the physical and chemical properties of the soil compared to other methods of sterilization. The objective of our research was to determine if the absence of viable microorganisms significantly affected the extent of chromium reduction in a reducing soil. Our hypothesis was that if geochemical reduction pathways dominated the system then soil sterilization should have little to no effect on the amount and rate of chromium that was reduced. However, if the reduction of chromium in these soils was a synergistic process then significantly different amounts of chromium should be reduced in the sterilized versus non-sterilized samples.

Mission Relevance: The research described within this report is an integral part of ERSD's Strategic Plan, which addresses "those fundamental issues where basic research can have a significant impact on characterization, assessment, cleanup, and stewardship at DOE sites" (DOE 2006). The research provides new experimental knowledge and information in previously unexplored areas of microbial vs. geochemical metal reduction rates and mechanisms that control *in situ* immobilization. Since geochemical reduction rates are often slow under conditions where metal reducing bacteria thrive, knowledge concerning the significance of geochemical vs. microbial metal reduction rates and mechanisms is imperative toward decision making strategies concerning metal immobilization strategies *in situ*. The research identifies various strategies to stimulate indigenous microorganisms that target contaminant metal reduction and immobilization and compares this to systems where microorganisms are absent. This fundamental information will have significant impact on groundwater remediation decisions at DOE sites where redox sensitive metals and radionuclides are present (e.g. Hanford, Oak Ridge, and Savannah River). The research also supports the ERSD and EM mission of long-term isolation, *in situ* remediation, and stewardship of contaminated sites. Further, this research combines DOE's commitment to environmental restoration with its commitment to major user facilities (e.g. SSRL, APS, EMSL).

Progress and Implications: This report summarizes research after 3y of a 3y project. Sterilized and non-sterilized soil columns were amended with carbon sources to elucidate the potential for geochemical and biological Cr(VI) reduction. Soil sterilization was achieved via exposure to γ -irradiation which causes fewer changes in the physical and chemical properties of the soil compared to other methods of sterilization. Cr(VI) breakthrough was significantly retarded in the lactate, ethanol, and acetate-amended

soils as noted by delayed breakthrough relative to a nonreactive Br tracer (Fig. 1). It was also observed that soils amended with lactate exhibited significantly more chromium retention relative to the other electron donor amended soils (Fig. 1). Soil sterilized by γ -irradiation retained limited amounts of Cr(VI) as exhibited by breakthrough profiles that were nearly identical to nonreactive Br (Fig. 2). These findings were consistent with batch microcosm investigation involving the same soil and electron donors (not shown).

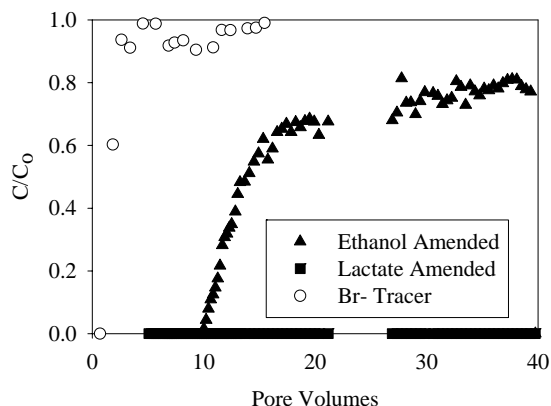


Figure 1. Cr(VI) breakthrough as a function of pore volume in lactate- and ethanol-amended soil columns. No Cr(VI) was measured in the lactate-amended column effluent after 40 pore volumes had passed through the column. Breakthrough of Cr(VI) in both amended columns was significantly retarded compared to the Br⁻ tracer.

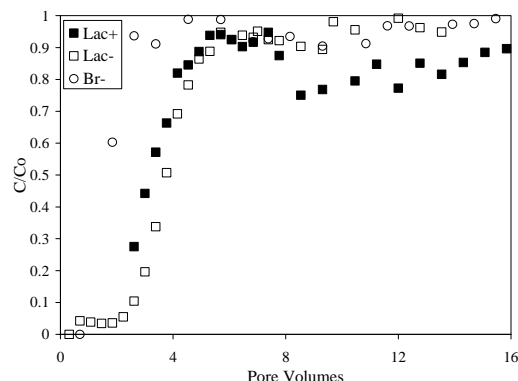


Figure 2. Cr(VI) breakthrough in sterilized soil columns with and without 25 mM lactate amendment. Breakthrough occurs only slightly delayed compared to the Br⁻ tracer indicating that Cr(VI) sorption or reduction in the absence of viable microorganisms is minimal.

To confirm the mechanism of Cr retention in the amended soils, X-ray Absorption Near Edge Structure (XANES) was used to interrogate the solid surface and determine the valence state of the Cr (Fig. . XANES analyses confirmed that all of the surface bound Cr was as Cr(III) has evidenced by the lack of a pre-edge Cr(VI) spectral peak. The results suggest that Cr(VI) is reduced to Cr(III) in these systems and the Cr(III) is either sorbed or precipitated on the solid phase. Since the reduction processes is significantly more prevalent in the non-sterilized soils, the results suggest that Cr(VI) reduction in these systems was at predominately biological in nature (Figs. 1 and 2).

To determine whether the reduction reaction was direct interaction of microorganisms with the Cr or whether it was an indirect biogenic reduction reaction, bacterial community analysis was performed on the non sterilized donor amended soils. Analyses of small subunit (ssu) rRNA genes amplified from the column sediments showed significantly different bacterial populations within the three amended soils (Fig. 4). Community analyses indicated that Cr(VI) reduction to Cr(III) was possibly coupled to the production of organic reductants by *Clostridiales*. This suggested that the reduction reaction was indirect biogenic reaction which was consistent with other related investigations in the literature.

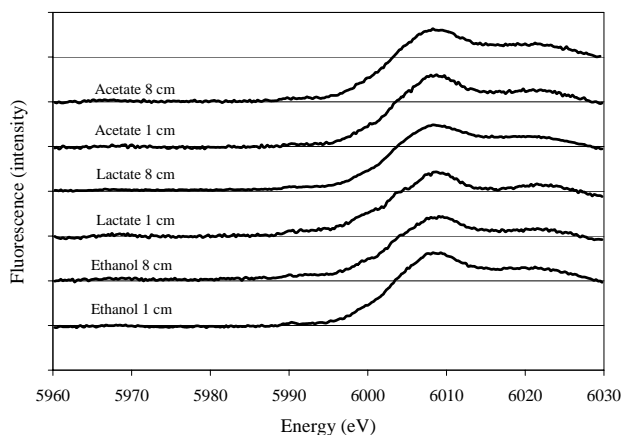
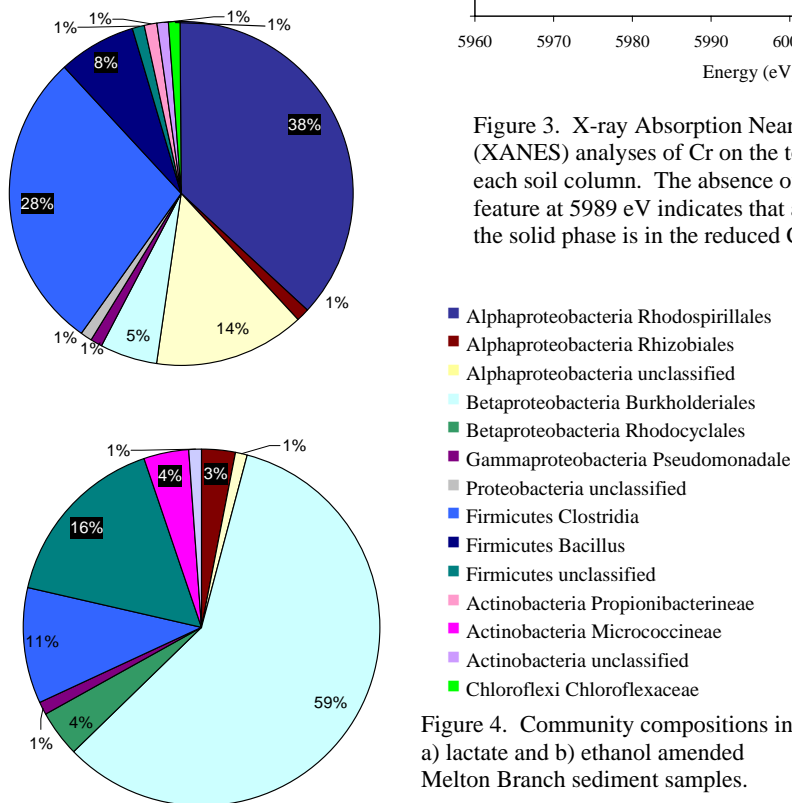


Figure 3. X-ray Absorption Near Edge Spectroscopy (XANES) analyses of Cr on the top and bottom 2 cm of each soil column. The absence of any Cr(VI) pre-edge feature at 5989 eV indicates that all Cr associated with the solid phase is in the reduced Cr(III) state.



The column and batch sorption/reduction experiments indicated that the uncontaminated saprolitic soils from the ORR had reducing capabilities that were not limited to geochemical pathways. Because sterilized soils did not sorb or reduce a measurable quantity of Cr(VI), we deduced that the indigenous microbial population played a key role in the amount and rate of Cr(VI) reduction that occurred under anaerobic conditions. In these soils, biological reduction proceeded at a faster rate than potential geochemical reduction when a suitable carbon source was provided. In the timescale of our experiments (~17 days) geochemical reduction in sterilized soils did not occur to any measurable extent in either batch or column studies.

The microbial population identified from the ethanol- and lactate-amended sediments indicated that, despite purging with CO₂, aerobic pockets may have existed within the sediment columns; obligate aerobes (*Burkholderiales*) and obligate anaerobes (*Clostridiales*) coexisted in the ethanol- and lactate-amended columns (Fig 4). In the ethanol-amended sediment nearly 60% of the organisms analyzed had sequences closely resembling *Burkholderiales*. These results suggested that major differences in bacterial

communities were likely due to experimental setup rather than added carbon source, though it was also possible that the carbon source contributed to the bacterial diversity measured in these systems.

Based on the microbial populations identified in these sediments, direct reduction of Cr(VI) to Cr(III) was unlikely in our experiments because no known Cr(VI) reducers were immediately identified. Because more *Clostridiales* were present in the lactate-amended soil (which reduced significantly more chromium than the other amended sediments in both column and batch studies) it is surmised that indirect reduction of Cr(VI) may have occurred as a result of reduced iron or organic reductants produced by *Clostridiales*. The increased number of *Clostridiales* in the lactate amended soil may be due to soil heterogeneity, fewer aerobic pockets in the soil column, or a greater affinity of *Clostridiales* to grow on the lactate source.

Effects of Sterilization on the Sorption of U(VI) onto Natural Sediments from the Oak Ridge Reservation

A series of experiments were completed to determine the effects of soil sterilization on various soil chemical properties including U(VI) sorption, soil pH, natural organic matter (NOM), cation exchange capacity (CEC), and iron oxidation state. Soils under investigation were a saprolitic sequence of interbedded weathered shale and limestone collected from the Oak Ridge Reservation (ORR). Sediments were sterilized by either steam sterilization at 121°C or by γ -irradiation using a Cobalt-60 source. Subsamples of sediments were pretreated with the reductant solution dithionate-citrate-bicarbonate (DCB) and/or H_2O_2 to remove reducible Fe(III) oxides and NOM. Results indicated that sterilization by γ -irradiation caused a measurable increase in the amount of U(VI) that was sorbed to the sediment. Experiments completed on DCB and H_2O_2 -treated samples indicated that the iron oxide minerals and NOM accounted for the majority of U(VI) sorption and that changes due to γ -irradiation of these phases resulted in increased U(VI) sorption. Results from Mössbauer spectroscopy indicated a decrease in the amount of ferric iron associated with goethite and a small increase in the amount of reduced iron in silicate minerals post-irradiation (Fig. 5). Our results suggest that sterilization by γ -irradiation induces iron reduction that can lead to increased sorption of U(VI) on these sediments.

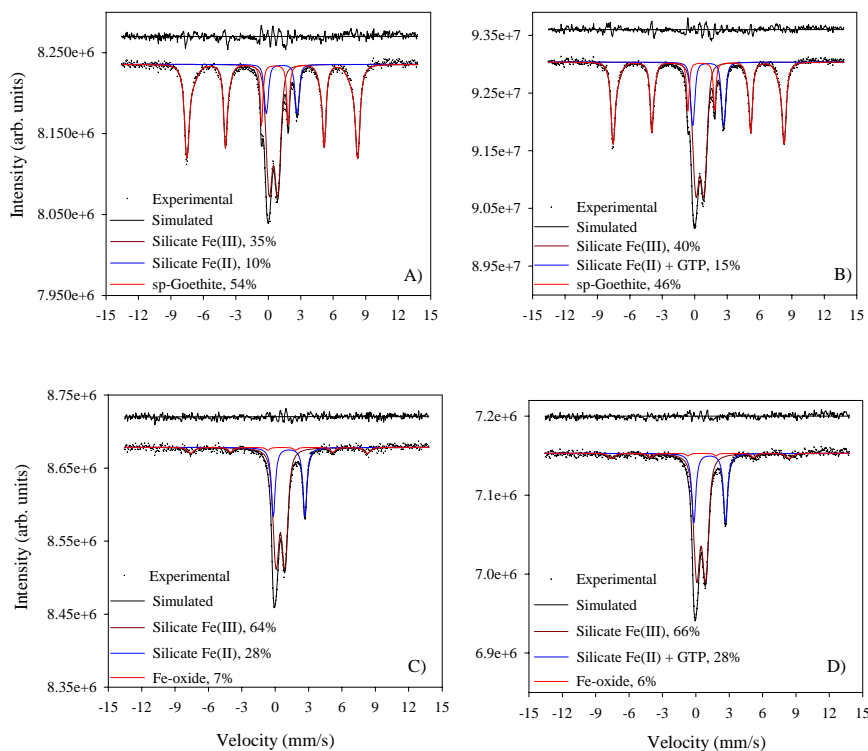


Figure 5 ^{57}Fe transmission Mössbauer 12K spectra of A) non-sterile untreated soil, B) g-irradiated untreated soil, C) non-sterile DCB-treated soil and D) irradiated DCB-treated soil. Results indicate that g-induced iron reduction probably occurs in the iron oxide minerals and not in iron silicate minerals. Duplicate samples not shown. GTP=goethite transformation product.

Peer-reviewed manuscripts and abstracts/presentations:

Bank, T.L., T.A. Vishnivetskaya, M.A. Ginder-Vogel, S. Fendorf, M. E. Baldwin, and P.M. Jardine. Elucidating Biogeochemical Reduction of Chromate via Carbon Amendments and Soil Sterilization. *Geomicrobiology Journal* (in press).

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Bank, T.L., P.M. Jardine, M. Ginder-Vogel, S. E. Fendorf, and M. E. Baldwin. 2005. Discerning Geochemical and Biogeochemical Metal Reduction through Gamma Sterilization. 15th Annual Goldschmidt Conference, May 20-25, Moscow, ID.

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